**STIC-ILL** NPL

**From:** Holleran, Anne
**Sent:** Wednesday, August 20, 2003 12:55 PM
**To:** STIC-ILL
**Subject:** refs. for 09/094,921

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| 1. | Ruf | Blood (2001) 98(8): 2526-2534 |
| 2. | Talac | J. Biological Regulators and Homeostatic Agents (2001) 14(30): 175-181 |
| 3. | Zeidler | J. Immunology (1999) 163(3): 1246-1252 |
| 4. | Mocikat | Cancer Res. (1997) 57(12): 2346-2349 |
| 5. | Heijnen | Cancer Immunology, Immunotherapy (1997) 45(3-4): 166-170 |
| 6. | Haagen | J. Immunology (1995) 154(4): 1852-1860 |
| 7. | Hazra | Nuclear Medicine Communications (1995) 16(2): 66-75 |
| 8. | Francois | J. Immunology (1993) 150(10): 4610-4609 |
| 9. | Beun | J. Immunotherapy (1993) 13(4): 223-231 |
| 10. | Brissinck | Drugs of the Future (1992) 17(11): 1003-1010 |
| 11. | Clark | J. Nat. Cancer Inst. (1987) 79(6): 1393-1401 |

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**STIC-ILL**

N08/20

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4607 11

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| 1. | Ruf | Blood (2001) 98(8): 2526-:534 |
| 2. | Talac | J. Biological Regulators a: d Homeostatic Agents (2001) 14(30): 175-181 |
| 3. | Zeidler | J. Immunology (1999) 16: (3): 1246-1252 |
| 4. | Mocikat | Cancer Res. (1997) 57(1:): 2346-2349 |
| 5. | Heijnen | Cancer Immunology, Imm nnotherapy (1997) 45(3-4): 166-170 |
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| 9. | Beun | J. Immunotherapy (1993) 13(4): 223-231 |
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| 5. | Heijnen | Cancer Immunology, Immunotherapy (1997) 45(3-4): 166-170 |
| 6. | Haagen | J. Immunology (1995) 154(4): 1852-1860 |
| 7. | Hazra | Nuclear Medicine Communications (1995) 16(2): 66-75 |
| 8. | Francois | J. Immunology (1993) 150(10): 4610-4609 |
| 9. | Beun | J. Immunotherapy (1993) 13(4): 223-231 |
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| 5. | Heijnen | Cancer Immunology, Immunotherapy (1997) 45(3-4): 166-170 |
| 6. | Haagen | J. Immunology (1995) 154(4): 1852-1860 |
| 7. | Hazra | Nuclear Medicine Communications (1995) 16(2): 66-75 |
| 8. | Francois | J. Immunology (1993) 150(10): 4610-4609 |
| 9. | Beun | J. Immunotherapy (1993) 13(4): 223-231 |
| 10. | Brissinck | Drugs of the Future (1992) 17(11): 1003-1010 |
| 11. | Clark | J. Nat. Cancer Inst. (1987) 79(6): 1393-1401 |

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**STIC-ILL** RM270, J6

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**To:** STIC-ILL
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| 2. | Talac | J. Biological Regulators and Homeostatic Agents (2001) 14(30): 175-181 |
| 3. | Zeidler | J. Immunology (1999) 163(3): 1246-1252 |
| 4. | Mocikat | Cancer Res. (1997) 57(12): 2346-2349 |
| 5. | Heijnen | Cancer Immunology, Immunotherapy (1997) 45(3-4): 166-170 |
| 6. | Haagen | J. Immunology (1995) 154(4): 1852-1860 |
| 7. | Hazra | Nuclear Medicine Communications (1995) 16(2): 66-75 |
| 8. | Francois | J. Immunology (1993) 150(10): 4610-4609 |
| 9. | Beun | J. Immunotherapy (1993) 13(4): 223-231 |
| 10. | Brissinck | Drugs of the Future (1992) 17(11): 1003-1010 |
| 11. | Clark | J. Nat. Cancer Inst. (1987) 79(6): 1393-1401 |

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| 3. | Zeidler | J. Immunology (1999) 163(3): 1246-1252 |
| 4. | Mocikat | Cancer Res. (1997) 57(12): 2346-2349 |
| 5. | Heijnen | Cancer Immunology, Immunotherapy (1997) 45(3-4): 166-170 |
| 6. | Haagen | J. Immunology (1995) 154(4): 1852-1860 |
| 7. | Hazra | Nuclear Medicine Communications (1995) 16(2): 66-75 |
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| 11. | Clark | J. Nat. Cancer Inst. (1987) 79(6): 1393-1401 |

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L6 ANSWER 1 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:376886 CAPLUS
DOCUMENT NUMBER: 138:384151
TITLE: Anti-human CD40 antibodies and fragments for diagnosis and therapy of cancer
INVENTOR(S): Bedian, Vahe; Gladue, Ronald P.; Corvalan, Jose; Jia, Xiao-Chi; Feng, Xiao
PATENT ASSIGNEE(S): Pfizer Products Inc., USA; Abgenix, Inc.
SOURCE: PCT Int. Appl., 177 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003040170 | A2 | 20030515 | WO 2002-US36107 | 20021108 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-348980P P 20011109

AB The present invention relates to antibodies and antigen-binding portions thereof that specifically bind to CD40, preferably human CD40, and that function as CD40 agonists. The invention also relates to human anti-CD40 antibodies and antigen-binding portions thereof. The invention also relates to antibodies that are humanized, chimeric, ***bispecific*** , derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain Igs derived from human anti-CD40 antibodies and nucleic acid mols. encoding such Igs. The present invention also relates to methods of making human anti-CD40 antibodies, compns. comprising these antibodies and methods of using the antibodies and compns. for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid mols. encoding the heavy and/or light Ig mols. that comprise the human anti-CD40 antibodies. The invention also relates to transgenic animals comprising nucleic acid mols. of the present invention.

L6 ANSWER 2 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:435072 CAPLUS
DOCUMENT NUMBER: 139:21017
TITLE: Prostate-associated protease HUPAP, cDNA and antibodies for prognosis, diagnosis and treatment of prostate cancer
INVENTOR(S): Spancake, Kimberly M.; Bandman, Olga; Lal, Preeti G.
PATENT ASSIGNEE(S): Incyte Genomics, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 42 pp., Cont.-in-part of U.S. Ser. No. 988,975.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 2003103981 | A1 | 20030605 | US 2002-235699 | 20020904 |
| US 6043033 | A | 20000328 | US 1997-807151 | 19970227 |

US 6350448 B1 20020226 US 2000-478957 20000107
US 2002119531 A1 20020829 US 2001-988975 20011119
PRIORITY APPLN. INFO.: US 1997-807151 A3 19970227
US 2000-478957 A2 20000107
US 2001-988975 A2 20011119
AB The invention provides a cDNA which encodes a human prostate-assocd. protease, or kallikrein designated as HUPAP, differentially expressed in prostate cancer. It also provides for the use of the cDNA, fragments, complements, and variants thereof and of the encoded protein, portions thereof and antibodies thereto for diagnosis and treatment of prostate cancer. The invention addnl. provides expression vectors and host cells for the prodn. of the protein and a transgenic model system.

L6 ANSWER 3 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:488616 CAPLUS
DOCUMENT NUMBER: 139:67777
TITLE: Generation of genetically modified vertebrate precursor lymphocytes for production of ***antibody*** , antigen receptor, heterologous binding protein or fragment
INVENTOR(S): Grawunder, Ulf; Melchers, Georg Friedrich
PATENT ASSIGNEE(S): Germany
SOURCE: Eur. Pat. Appl., 111 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 1321477 | A1 | 20030625 | EP 2001-130805 | 20011222 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.: EP 2001-130805 20011222
AB The present invention generally relates to the fields of genetic engineering and ***antibody*** prodn. In particular, it relates to the generation of genetically modified vertebrate precursor lymphocytes that have the potential to differentiate into more mature lymphoid lineage cells, and to the use thereof for the prodn. of any heterologous ***antibody*** or binding protein. Retroviral vector pLIB-bcl2-IRES-hygroB was constructed for overexpression of Bcl2 gene in murine. Long term proliferating murine precursor B cells with inactivated endogenous Ig. heavy and light chain gene loci were prepd. and used to generate human Ig.-producing murine precursor B cells.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:185183 CAPLUS
DOCUMENT NUMBER: 136:246395
TITLE: Human antibodies against Pseudomonas aeruginosa lipopolysaccharide derived from transgenic ***mouse***
INVENTOR(S): Schreiber, John R.; Kamboj, Kulwant Kauer
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 84 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002020619 | A2 | 20020314 | WO 2001-US28019 | 20010907 |
| WO 2002020619 | A3 | 20030123 | | |

WO 2002020619 C2 20030417
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
AU 2001088866 A5 20020322 AU 2001-88866 20010907
EP 1319025 A2 20030618 EP 2001-968629 20010907
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.: US 2000-230640P P 20000907
US 2001-259472P P 20010103
WO 2001-US28019 W 20010907

AB The authors disclose human antibodies produced in non-human animals (XenoMouse) that specifically bind to Pseudomonas aeruginosa lipopolysaccharide (LPS). In one example, the authors prep. and characterize a human monoclonal ***antibody*** S20 (IgG2,.kappa.) that reacts specifically with the O-side chain of P. aeruginosa serotype 06ad polysaccharide. The S20 ***antibody*** was shown to mediate complement-dependent phagocytosis of P. aeruginosa by human polymorphonuclear leukocytes and to provide protection of neutropenic mice from fatal sepsis.

L6 ANSWER 5 OF 49 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001561924 MEDLINE
DOCUMENT NUMBER: 21471979 PubMed ID: 11588051
TITLE: Induction of a long-lasting antitumor immunity by a trifunctional ***bispecific*** ***antibody*** .
AUTHOR: Ruf P; Lindhofer H
CORPORATE SOURCE: Clinical Cooperation Group Bispecific Antibodies of the Department of Otorhinolaryngology, Ludwig Maximilians University, Munich, Germany.
SOURCE: BLOOD, (2001 Oct 15) 98 (8) 2526-34.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011022
Last Updated on STN: 20020122
Entered Medline: 20011205

AB ***Bispecific*** antibodies (bsAbs) can efficiently mediate tumor cell killing by redirecting preactivated or costimulated T cells to disseminated tumor cells, especially in a minimal residual disease situation. This study demonstrates that the trifunctional bsAb BiLu is able to kill tumor cells very efficiently without any additional costimulation of effector cells in vitro and in vivo. Remarkably, this bsAb also induces a long-lasting protective immunity against the targeted syngeneic ***mouse*** tumors (B16 melanoma and A20 B-cell lymphoma, respectively). A strong correlation was observed between the induction of a humoral immune response with tumor-reactive antibodies and the survival of mice. This humoral response was at least in part tumor specific as shown in the A20 model by the detection of induced anti-idiotype antibodies. Both the survival of mice and antitumor titers were significantly diminished when F(ab')(2) fragments of the same bsAb were applied, demonstrating the importance of the Fc region in this process. With the use of T-cell depletion, a contribution of a cellular antitumor response could be demonstrated. These results reveal the necessity of the Fc region of the bsAb with its potent immunoglobulin subclass combination ***mouse*** immunoglobulin G2a (IgG2a) and ***rat*** IgG2b. The antigen-presenting system seems to be crucial for achieving an efficient tumor cell killing and induction of long-lasting antitumor immunity.

Hereby, the recruitment and activation of accessory cells by the intact bsAb is essential.

L6 ANSWER 6 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:152839 BIOSIS
DOCUMENT NUMBER: PREV200200152839
TITLE: Isolation of hematopoietic progenitor cells and mature cell subsets from rhesus monkey bone marrow and peripheral blood by negative selection.
AUTHOR(S): Wognum, Albertus W. (1); Visser, Trudy P.; Peters, Kathelijn; Thomas, Terry E. (1); Wagemaker, Gerard
CORPORATE SOURCE: (1) StemCell Technologies, Vancouver, BC Canada
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 340b-341b. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The use of nonhuman primates as large animal models for preclinical studies of human hematopoiesis and therapy requires identification and isolation of specific hematopoietic and lymphoid cell populations from simian blood, bone marrow and other tissues. Isolation parameters for human stem cells cannot be readily applied to monkeys, as most antibodies against human cells do not recognize monkey cells or target different cell populations. In addition, the cellular distribution of markers for CD34+ subsets in humans, e.g., CD38, HLA-DR, Thy-1 or c-Kit, is different on simian cells and these markers cannot be readily applied to identification and isolation of monkey hematopoietic cells. In this study we have used targeted depletion of specific cell populations to isolate primitive hematopoietic cells and other cells from rhesus monkey bone marrow and blood. A panel of antibodies was first identified that recognize monkey lymphoid cells (i.e., CD2, CD3, CD4, CD8, CD20) myelomonocytic cells (CD16, CD56, CD11b, CD66e) and erythroid cells. These antibodies were then conjugated to anti-dextran antibodies by noncovalent crosslinking with ***rat*** monoclonal antibodies against ***mouse*** IgG1 to form ***bispecific*** tetrameric ***antibody*** complexes (TAC). Unwanted cells were then depleted by incubation with specific anti-cell/anti-dextran TACs and dextran-coated magnetic colloid, followed by passage over a magnetic column, using the StemSepTM methodology. T cells were effectively purified with a cocktail of TACs against B-lymphocytes, NK cells, monocytes and granulocytes (CD11b, CD14, CD16, CD20, CD56, CD66e) (97% purity and 49% recovery of CD3+ T cells). CD4+ and CD8+ T cell subsets were isolated with similar purity and recovery by adding anti-CD8 TAC or anti-CD4 TAC to the T cell enrichment cocktail, to deplete CD8+ or CD4+ T cells, respectively. Depletion of lineage-positive bone marrow cells was then performed using TACs against CD2, CD3, CD4, CD8, CD11b, CD14, CD16, CD20 and monkey erythrocytes. Clonogenic growth of monkey progenitor cells was evaluated in methylcellulose media supplemented with human IL-3, GM-CSF and EPO, or IL-3, GM-CSF, G-CSF, IL-6, SCF and EPO. Mature and differentiating precursor cells for major blood cell lineages were effectively depleted by the immunomagnetic procedure. However, the recovery of rhesus monkey cells that expressed CD34 was 6.8-19.5% (dependent on the depletion cocktail used), which is lower than after positive selection using anti-CD34 antibodies. This was attributed to co-expression of lineage markers, in particular myelomonocytic markers CD11b, CD14, CD16 and CD56, on variable fractions (2 to apprx50%) of rhesus monkey CD34+ cells, indicating that CD34 is less useful as stem cell and progenitor marker for monkey cells than for human cells. Approaches to enrich immature hematopoietic cells by systematic depletion of lineage-committed and differentiated progenitor cells are essential to identify the most primitive cells, including those stem cell subsets that do not express CD34 or other known stem cell markers, and will be important for preclinical research on stem cell plasticity, stem cell expansion, transplantation and gene therapy.

L6 ANSWER 7 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2001136336 EMBASE
TITLE: Current perspectives of ***bispecific*** ***antibody*** -based immunotherapy.
AUTHOR: Talac R.; Nelson H.
CORPORATE SOURCE: Dr. H. Nelson, Division of Colon/Rectal Surgery, Mayo Clinic, 200 First Street SW, Rochester, MN 55902, United States. nelson.heidi@mayo.edu
SOURCE: Journal of Biological Regulators and Homeostatic Agents, (2001) 14/3 (175-181).
Refs: 67
ISSN: 0393-974X CODEN: JBRAER
COUNTRY: Italy
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The field of ***bispecific*** antibodies is an evolving field of research that has increasing clinical appeal. The fusion of two antibodies or ***antibody*** fragments introduced a new way to override natural specificity of T cell and induce effector responses against tumor targets in MHC-unrestricted manner. Initial experiences with ***bispecific*** antibodies demonstrate both the promise for and limitations of this anti-cancer strategy. Significant body of work has shown that ***bispecific*** antibodies have potential to induce T cell mediated anti-tumor responses in pre-clinical models. However, Immunotherapy with ***bispecific*** antibodies in humans has yet to prove its value in clinical settings. In addition, the production of high-quality ***bispecific*** antibodies for clinical applications, the optimal size and avidity of ***bispecific*** antibodies, and in vivo T cell pre-activation remain critical issues. In this review, we summarize recent progress in ***bispecific*** ***antibody*** -based immunotherapy and address essential aspects of this anti-cancer strategy.

L6 ANSWER 8 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:227691 CAPLUS
DOCUMENT NUMBER: 132:250020
TITLE: ***Bispecific*** and trispecific antibodies which specifically react with inducible surface antigens as operational target structures
INVENTOR(S): Lindhofer, Horst
PATENT ASSIGNEE(S): Germany
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000018806 | A1 | 20000406 | WO 1999-EP7095 | 19990922 |
| W: CA, JP, US | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| DE 19859110 | A1 | 20000413 | DE 1998-19859110 | 19981221 |

PRIORITY APPLN. INFO.: DE 1998-19844157 A 19980925
DE 1998-19859110 A 19981221
AB According to the invention, an intact ***bispecific*** or trispecific ***antibody*** is provided which comprises at least the following properties: (a) binding to a T cell; (b) binding to at least one antigen on a target cell; (c) binding by the Fc portion thereof (in ***bispecific*** antibodies) or by a third specificity (in trispecific antibodies). The antigen can be induced and is not found on the target cell in a non-induced state (normal state) or it exists in a low no. that

is insufficient to destroy the target cell. The use of these antibodies for immunotherapy of tumors and infections is discussed.
REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2000117843 EMBASE
TITLE: Advances in the use of monoclonal antibodies in cancer radiotherapy.
AUTHOR: Govindan S.V.; Goldenberg D.M.; Hansen H.J.; Griffiths G.L.
CORPORATE SOURCE: S.V. Govindan, Immunomedics Inc., Corporate Headquarters, 300 American Road, Morris Plains, NJ 07950, United States. sgovindan@immunomedics.com
SOURCE: Pharmaceutical Science and Technology Today, (1 Mar 2000) 3/3 (90-98).
Refs: 63
ISSN: 1461-5347 CODEN: PSTTF8
PUBLISHER IDENT.: S 1461-5347(00)00241-8
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 014 Radiology
016 Cancer
023 Nuclear Medicine
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The use of monoclonal antibodies (MAbs) as radiation carriers in argeted radiotherapy of cancers has produced striking clinical responses in hematologic diseases, such as non-Hodgkin's lymphoma. Novel strategies are currently being examined in an effort to improve efficacy in solid tumor therapies. Two of these strategies involve minimizing the systemic toxicity of a circulating radionuclide via 'pretargeting', and the sensitization of tumors to radiation by combination therapy with radiosensitizing drugs. Advances made in radiolabeling chemistries and in the use of alpha-particle emitters can also improve utility. Clinical evidence suggests that radioimmunotherapy may be best applied in minimal-disease and adjuvant settings in combination with other cancer therapy modalities. Copyright (C) 2000 Elsevier Science Ltd.

L6 ANSWER 10 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:561564 CAPLUS
DOCUMENT NUMBER: 131:183874
TITLE: Method for producing heterologous ***bispecific*** antibodies
INVENTOR(S): Lindhofer, Horst; Thierfelder, Stephan
PATENT ASSIGNEE(S): GSF--Forschungszentrumfur Umweltund Gesundheit, Germany
SOURCE: U.S., 10 pp., Cont.-in-part of PCT Ser. No. EP95/01850.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 5945311 | A | 19990831 | US 1996-758430 | 19961129 |
| DE 4419399 | C1 | 19950309 | DE 1994-4419399 | 19940603 |
| WO 9533844 | A1 | 19951214 | WO 1995-EP1850 | 19950516 |

W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRIORITY APPLN. INFO.: DE 1994-4419399 A 19940603
WO 1995-EP1850 A2 19950516
AB In a method for producing heterologous bi-specific antibodies, a ***quadroma*** is provided which is fused from hybridomas one of which generates antibodies that have an affinity to the binding site of protein

A and another of which generates antibodies that have a weaker or no affinity to the binding domain of protein A, by multiplying and cultivating the quadromas and by eluting the bi-specific antibodies in a pH range at least 0.5 units above the pH value at which the antibodies with greater affinity to the binding domain of protein A are still bonded. The first protein A-binding portion is derived from ***mouse*** or human or humanized IgG1, IgG2 IgG4 or ***rat*** IgG2c; and the second non-protein A-binding portion is derived from ***rat*** IgG1, IgG2a, IgG2b, IgG3 or human or humanized IgG3.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 49 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1999343730 MEDLINE
DOCUMENT NUMBER: 99343730 PubMed ID: 10415020
TITLE: Simultaneous activation of T cells and accessory cells by a new class of intact ***bispecific*** ***antibody*** results in efficient tumor cell killing.
AUTHOR: Zeidler R; Reisbach G; Wollenberg B; Lang S; Chaubal S; Schmitt B; Lindhofer H
CORPORATE SOURCE: Clinical Cooperation Group Bispecific Antibodies, Department of Otorhinolaryngology, Ludwig-Maximilians-University, Munich, Germany.
SOURCE: JOURNAL OF IMMUNOLOGY, (1999 Aug 1) 163 (3) 1246-52.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990820
Last Updated on STN: 19990820
Entered Medline: 19990812

AB ***Bispecific*** Abs (bsAb) are promising immunological tools for the elimination of tumor cells in minimal residual disease situations. In principle, they target an Ag on tumor cells and recruit one class of effector cell. Because immune reactions in vivo are more complex and are mediated by different classes of effector cell, we argue that conventional bsAb might not yield optimal immune responses at the tumor site. We therefore constructed a bsAb that combines the two potent effector subclasses ***mouse*** IgG2a and ***rat*** IgG2b. This ***bispecific*** molecule not only recruits T cells via its one binding arm, but simultaneously activates FcgammaR+ accessory cells via its Fc region. We demonstrate here that the activation of both T lymphocytes and accessory cells leads to production of immunomodulating cytokines like IL-1beta, IL-2, IL-6, IL-12, and DC-CK1. Thus this new class of bsAb elicits excellent antitumor activity in vitro even without the addition of exogenous IL-2, and therefore represents a totally self-supporting system.

L6 ANSWER 12 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 1999201649 EMBASE
TITLE: Mast cell stimulation by co-clustering the type I Fc.epsilon.-receptors with mast cell function-associated antigens.
AUTHOR: Schweitzer-Stenner R.; Engelke M.; Licht A.; Pecht I.
CORPORATE SOURCE: R. Schweitzer-Stenner, Institut fur Experimentelle Physik, Universitat Bremen, 28359 Bremen, Germany.
stenner@theo.physik.uni-bremen.de
SOURCE: Immunology Letters, (3 May 1999) 68/1 (71-78).
Refs: 20
ISSN: 0165-2478 CODEN: IMLED6
PUBLISHER IDENT.: S 0165-2478(99)00032-2
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The secretory response of ***rat*** mucosal-type mast cells (line RBL 2H3) to stimuli produced by clustering or co-clustering two of its membranal components; the type I Fc.epsilon. receptor and the mast cell function associated antigen (MAFA) was investigated. The primary reagents employed for this purpose were Fab fragments of the monoclonal antibodies J17 and G63 specific to the above respective proteins. The Fabs were then aggregated by F(ab')2 fragments of ***mouse*** IgG specific goat antibodies. This reaction was assumed to yield predominantly three different bivalent clustering reagents. Namely, dimers of the Fc.epsilon.RI specific (J17-Fab)2; dimers of the MAFA specific, (G63-Fab)2 and ***bispecific*** (J17-Fab-G63-Fab) dimers. The observed cellular secretory response was analyzed by employing a model which accounts for the clustering and co-clustering of Fc.epsilon.RIs and MAFAs by the above protocols. Results of this analysis provided evidence that at least some of the MAFA molecules are physically associated with the Fc.epsilon.RI. As a consequence, clustering of MAFA and Fc.epsilon.RI by ***bispecific*** J17-Fab-G63-Fab dimers induces secretion at comparatively low concentrations of these reagents, though with a significantly lower maximal response than that caused by the respective monospecific reagent (J17-Fab)2. This result most likely reflects the inhibitory capacity of MAFA-Fc.epsilon.RI interaction.

L6 ANSWER 13 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:12374 CAPLUS
DOCUMENT NUMBER: 130:51356
TITLE: Method of ex vivo immunizing using heterologous intact ***bispecific*** and/or trispecific antibodies
INVENTOR(S): Lindhofer, Horst; Kolb, Hans-Jochem; Zeidler, Reinhard; Bornkamm, Georg
PATENT ASSIGNEE(S): Gsf-Forschungszentrum Fur Umwelt Und Gesundheit, Gmbh, Germany
SOURCE: Eur. Pat. Appl., 19 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 885614 | A2 | 19981223 | EP 1998-110972 | 19980616 |
| EP 885614 | A3 | 19990113 | | |
| EP 885614 | B1 | 20000927 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| DE 19725586 | A1 | 19981224 | DE 1997-19725586 | 19970617 |
| DE 19725586 | C2 | 19990624 | | |
| US 2002009430 | A1 | 20020124 | US 1998-94921 | 19980615 |
| AT 196607 | E | 20001015 | AT 1998-110972 | 19980616 |
| JP 11071288 | A2 | 19990316 | JP 1998-170389 | 19980617 |
| HK 1017270 | A1 | 20010112 | HK 1999-102534 | 19990611 |

PRIORITY APPLN. INFO.: DE 1997-19725586 A 19970617
AB The invention describes a method for ex vivo immunization of human and animal with the following steps: (a) isolation of autologous tumor cells; (b) treatment of tumor cells to prevent their survival after reinfusion; (c) incubation of treated tumor cells with intact heterologous ***bispecific*** and or trispecific antibodies. The antibodies have the following properties: binding to T-cells, binding to an antigen from the tumor cells, binding through its Fc fragment (by ***bispecific*** antibodies) or through a third specificity (by trispecific antibodies) to Fc-pos. cells.

L6 ANSWER 14 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:176147 CAPLUS
DOCUMENT NUMBER: 128:216369
TITLE: Bi- and trispecific antibodies for induction of tumor immunity

INVENTOR(S): Lindhofer, Horst; Kolb, Hans-Jochem; Thierfelder, Stefan
PATENT ASSIGNEE(S): GSF-Forschungszentrum fuer Umwelt und Gesundheit G.m.b.H. Neuherberg, Germany
SOURCE: Ger. Offen., 18 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| DE 19710497 | A1 | 19980305 | DE 1997-19710497 | 19970313 |
| DE 19710497 | C2 | 19980709 | | |
| DE 19649223 | A1 | 19980305 | DE 1996-19649223 | 19961127 |
| DE 19649223 | C2 | 19980730 | | |
| EP 826695 | A1 | 19980304 | EP 1997-115188 | 19970902 |
| EP 826695 | B1 | 20011212 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| EP 826696 | A1 | 19980304 | EP 1997-115190 | 19970902 |
| EP 826696 | B1 | 20020529 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| AT 210682 | E | 20011215 | AT 1997-115188 | 19970902 |
| AT 218143 | E | 20020615 | AT 1997-115190 | 19970902 |
| ES 2169299 | T3 | 20020701 | ES 1997-115188 | 19970902 |
| ES 2176574 | T3 | 20021201 | ES 1997-115190 | 19970902 |
| JP 10179151 | A2 | 19980707 | JP 1997-238745 | 19970903 |
| JP 3257970 | B2 | 20020218 | | |
| US 5985276 | A | 19991116 | US 1997-922966 | 19970903 |
| US 2002051780 | A1 | 20020502 | US 1997-923852 | 19970903 |
| US 6551592 | B2 | 20030422 | | |
| US 6210668 | B1 | 20010403 | US 1999-422878 | 19991021 |

PRIORITY APPLN. INFO.: DE 1996-19635743 A1 19960903
DE 1996-19648976 A1 19961126
DE 1996-19649223 A 19961127
DE 1997-19710497 A 19970313
US 1997-922966 A1 19970903

AB The invention concerns intact ***bispecific*** or trispecific antibodies, which can bind simultaneously to the T-cell receptor complex of T-cells, to tumor-assocd. antigens of a tumor cell, and through the Fc fragment of ***bispecific*** antibodies to Fc-receptor pos. cells. The use of these antibodies for induction of tumor immunity in humans and animals is discussed.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 15 OF 49 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1999018184 MEDLINE
DOCUMENT NUMBER: 99018184 PubMed ID: 9799527
TITLE: Real-time analysis of immunogen complex reaction kinetics using surface plasmon resonance.
AUTHOR: Yu Y Y; Van Wie B J; Koch A R; Moffett D F; Davis W C
CORPORATE SOURCE: Department of Chemical Engineering, Washington State University, Pullman, Washington 99164, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1998 Oct 15) 263 (2) 158-68.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981216

AB Real-time biospecific interactions of immunogens, measured via BIAcore, were used to verify qualitatively a biosensor design which relies on analyte binding competition reactions to open cross-linked receptor channels. The complexes of importance are: (1) cardiac troponin I (TnI) and monoclonal ***mouse*** anti-TnI IgG ***mAb*** 265, (2) TnI and ***bispecific*** antibodies (BsAbs) which on one end recognize TnI while the other end recognizes nicotinic acetylcholine receptors (nAChRs), (3) nAChRs and ***rat*** anti-nAChR IgG ***mAb*** 148, (4) nAChRs and BsAbs, (5) nAChRs and Fab'148-TnI biopolymers, and (6) ***mAb*** 265 and Fab-TnI biopolymers. A commonly used sensor chip, CM5, was employed to immobilize TnI by covalent amine coupling, while bilayer membrane-associated protein, nAChR, was noncovalently sequestered on a HPA sensor chip via hydrophobic adsorption of membrane lipids. The epitopes of membrane-bound nAChRs were still available to immunogens after being immobilized. Kinetic rate constants and affinities of these systems were calculated from BIAcore sensorgrams. The order of magnitude for dissociation rate constants of the BsAb/TnI linker complex and biopolymer/ ***mAb*** 265 complex is 10(-2) s-1, which provides an opportunity for competitive binding of free analyte in the sensing systems.


L6 ANSWER 16 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:281236 BIOSIS
DOCUMENT NUMBER: PREV199800281236
TITLE: Tumor localizing properties and boron targeting potential of a ***bispecific*** ***antibody*** .
AUTHOR(S): Liu, Liang; Barth, Rolf F. (1); Adams, Dianne M.; Yang, Weilian; Soloway, Albert H.; Reisfeld, Ralph A.
CORPORATE SOURCE: (1) Ohio State Univ., Dep. Pathol., 1645 Neil Ave., Columbus, OH 43210 USA
SOURCE: Larsson, B. [Editor]; Crawford, J. [Editor]; Weinreich, R. [Editor]. International Congress Series, (1997) No. 1132 PART 2, pp. 391-397. International Congress Series; Advances in neutron capture therapy, Vol. II, chemistry and biology.
Publisher: Elsevier Science Publishers B.V. PO Box 211, Sara Burgerhartstraat 25, 1000 AE Amsterdam, The Netherlands.
Meeting Info.: Seventh International Symposium on Neutron Capture Therapy for Cancer Zurich, Switzerland September 4-7,1996
ISSN: 0531-5131. ISBN: 0-444-82781-1.
DOCUMENT TYPE: Book; Conference
LANGUAGE: English

L6 ANSWER 17 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 97182205 EMBASE
DOCUMENT NUMBER: 1997182205
TITLE: Trioma-based vaccination against B-cell lymphoma confers long-lasting tumor immunity.
AUTHOR: Mocikat R.; Selmayr M.; Thierfelder S.; Lindhofer H.
CORPORATE SOURCE: R. Mocikat, GSF-Institut fur Immunologie, Marchioninistrasse 25, D-81377 Munchen, Germany. mocikat@gsf.de
SOURCE: Cancer Research, (1997) 57/12 (2346-2349).
Refs: 29
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
025 Hematology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A major goal of tumor immunotherapy is the induction of a systemic immune response against tumor antigens such as the tumor-specific immunoglobulin

idiotype (Id) expressed by lymphomas of the B-cell lineage. We describe an approach based on specific redirection of the tumor Id toward professional antigen-presenting cells (APCs), thereby overcoming the inefficient presentation on the parental transformed B cell. Lymphoma cells are fused to a xenogeneic hybridoma cell line that secretes an ***antibody*** against a surface molecule on APCs. Due to preferential assembly between heavy and light chains of antibodies of different species-origin, the resulting 'trioma' cells produce at high yield a ***bispecific*** ***antibody*** containing the lymphoma Id and the APC-binding arm, which redirects the Id to APCs. Processing and presentation of the Id will lead to T-cell activation. An absolute requirement for inducing a complete tumor protection was the immunization with ***antibody*** -secreting trioma cells as a cell-based vaccine instead of the soluble ***bispecific*** ***antibody*** . Tumor immunity was specific and long-lasting. Both CD4+ and CD8+ T cells were necessary for inducing tumor immunity.

L6 ANSWER 18 OF 49 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1998098154 MEDLINE
DOCUMENT NUMBER: 98098154 PubMed ID: 9435865
TITLE: Lysis of murine B lymphoma cells by transgenic phagocytes via a human Fc gamma RI x murine MHC class II ***bispecific*** ***antibody*** .
AUTHOR: Heijnen I A; Glennie M J; van de Winkel J G
CORPORATE SOURCE: Department of Immunology and Medarex Europe, University Hospital Utrecht, The Netherlands.
SOURCE: CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 166-70.
Journal code: 8605732. ISSN: 0340-7004.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206
Entered Medline: 19980129
AB The class I IgG receptor (Fc gamma RI) on cytotoxic effector cells has been reported to initiate destruction of tumour cells by effector cells in vitro. We are aiming at developing an immunocompetent model to evaluate the cytotoxic capacity of human Fc gamma RI for the rejection of tumour cells in vivo. Therefore, we recently generated a transgenic ***mouse*** strain expressing human Fc gamma RI on monocytes, macrophages, and neutrophils. In these mice, the human receptor is up-regulated by granulocyte-colony-stimulating factor (G-CSF) and is able to trigger cellular responses. Subsequently, in the present study the B cell lymphoma IIA1.6 cell line is selected as a tumour target, and a human Fc gamma RI-directed antitumour ***bispecific*** ***antibody*** (bsAb) is constructed and characterized. Fab' fragments of ***mAb*** 22, which bind hFc gamma RI at an epitope that is distinct from the ligand binding site, were chemically linked to Fab' fragments of ***rat*** anti-(mMHC class II antigens) ***mAb*** M5/114, yielding bsAb 22 x M5/114. This bsAb was able to bind simultaneously to hFc gamma RI and mMHC class II antigens in a dose-dependent fashion. Binding of 22 x M5/114 to Fc gamma RI was not inhibited in the presence of human IgG. It is important to note that, MHC-class-II-expressing IIA1.6 lymphoma cells were lysed by whole blood from G-CSF-treated transgenic mice in the presence of bsAb 22 x M5/114. No lysis by whole blood from non-transgenic mice or from transgenic animals that had not received G-CSF was observed. These results indicate that human Fc gamma RI is able to mediate lysis of murine IIA1.6 lymphoma cells by transgenic effector cells via bsAb 22 x M5/114. A trial with transgenic mice, evaluating the efficacy of these hFc gamma RI-directed bsAb in combination with G-CSF for treatment of IIA1.6 B cell lymphoma, is currently in progress.

L6 ANSWER 19 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 96053503 EMBASE

DOCUMENT NUMBER: 1996053503
TITLE: Rapid and reliable cloning of ***antibody*** variable regions and generation of recombinant single chain ***antibody*** fragments.
AUTHOR: Gilliland L.K.; Norris N.A.; Marquardt H.; Tsu T.T.; Hayden M.S.; Neubauer M.G.; Yelton D.E.; Mittler R.S.; Ledbetter J.A.
CORPORATE SOURCE: Sir William Dunn School of Pathology, University of Oxford, South Parks Road,Oxford OX1 3RE, United Kingdom
SOURCE: Tissue Antigens, (1996) 47/1 (1-20).
ISSN: 0001-2815 CODEN: TSANA2
COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Single chain ***antibody*** variable region fragments (sFv), by virtue of their size and method of construction are potentially useful as therapeutic reagents and as tools for exploring cell surface receptor function. sFv offer several advantages over the intact immunoglobulin molecule. For instance, they are expressed from a single transcript and can be molecularly linked to other proteins to generate ***bispecific*** sFv molecules or single-chain immunotoxins. The relatively small size of sFv is an advantage in allowing for easier penetrance into tissue spaces, and their clearance rate is exceedingly rapid. sFv are useful for gene therapy since they can be directed to a specific cellular localization and can be fused to retroviral env genes to control viral host range. To prepare sFv to murine and human leukocyte CD antigens, we devised a method for rapid cloning and expression that can yield functional protein within 2 - 3 weeks of RNA isolation from hybridoma cells. The variable regions were cloned by poly-G tailing the first strand cDNA followed by anchor PCR with a forward poly-C anchor primer and a reverse primer specific for constant region sequence. Both primers contain flanking restriction sites for insertion into PUC19. Sets of PCR primers for isolation of murine, hamster and ***rat*** VL and VH genes were generated. Following determination of consensus sequences for a specific VL and VH pair, the VL and VH genes were linked by DNA encoding an intervening peptide linker [usually (Gly4Ser)3] and the VL-link-VH gene cassettes were transferred into the pCDM8 mammalian expression vector. The constructs were transfected into COS cells and sFvs were recovered from spent culture supernatant. We have used this method to generate functional sFv to human CD2, CD3, CD4, CD8, CD28, CD40, CD45 and to murine CD3 and gp39, from hybridomas producing murine, ***rat*** , or hamster antibodies. Initially, the sFvs were expressed as fusion proteins with the hinge-CH2-CH3 domains of human IgG1 to facilitate rapid characterization and purification using goat anti-human IgG reagents or protein A. We also found that active sFv could be expressed with a small peptide .gtoreq. tag .gtoreq. or in a tail-less form. Expression of CD3 (G19 - 4) sFv tail-less or Ig tailed forms demonstrated increased cellular signalling activity and suggested that sFv have potential for activating receptors.

L6 ANSWER 20 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:498513 CAPLUS
DOCUMENT NUMBER: 122:237782
TITLE: Process for producing heterologous ***bispecific*** antibodies
INVENTOR(S): Lindhofer, Horst; Thiefelder, Stefan
PATENT ASSIGNEE(S): GSF = Forschungszentrum fuer Umwelt und gesundheit GmbH, Germany
SOURCE: Ger., 10 pp.
CODEN: GWXXAW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| DE 4419399 | C1 | 19950309 | DE 1994-4419399 | 19940603 |
| WO 9533844 | A1 | 19951214 | WO 1995-EP1850 | 19950516 |
| W: CA, JP, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 763128 | A1 | 19970319 | EP 1995-919458 | 19950516 |
| EP 763128 | B1 | 19991201 | | |
| R: AT, BE, CH, DE, DK, FR, GB, IT, LI, NL, SE | | | | |
| JP 09506001 | T2 | 19970617 | JP 1995-500228 | 19950516 |
| AT 187176 | E | 19991215 | AT 1995-919458 | 19950516 |
| JP 3400457 | B2 | 20030428 | JP 1996-500228 | 19950516 |
| US 5945311 | A | 19990831 | US 1996-758430 | 19961129 |

PRIORITY APPLN. INFO.: DE 1994-4419399 A 19940603
WO 1995-EP1850 W 19950516

AB A process is described for producing heterologous ***bispecific*** IgG antibodies, or quadromas, from 2 fused hybridomas. One of the hybridomas produces antibodies with an affinity for protein A, and the other produces antibodies with little or no affinity for protein A. Thus, a ***quadroma*** was produced which included an anti- ***mouse*** CD3 ***rat*** ***antibody*** of the IgG2b subclass, and an anti- ***mouse*** Thy-1.2 ***mouse*** ***antibody*** of the IgG2a subclass.

L6 ANSWER 21 OF 49 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 95138530 MEDLINE
DOCUMENT NUMBER: 95138530 PubMed ID: 7836769
TITLE: Interaction of human monocyte Fc gamma receptors with ***rat*** IgG2b. A new indicator for the Fc gamma RIIa (R-H131) polymorphism.
AUTHOR: Haagen I A; Geerars A J; Clark M R; van de Winkel J G
CORPORATE SOURCE: Department of Immunology, University Hospital Utrecht, The Netherlands.
SOURCE: JOURNAL OF IMMUNOLOGY, (1995 Feb 15) 154 (4) 1852-60. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950314
Last Updated on STN: 19950314
Entered Medline: 19950227

AB ***Rat*** mAbs receive considerable interest for immunologic intervention in man. The ***rat*** IgG2b isotype has previously been found to be optimally active both in vivo and in vitro. We found that both a ***rat*** IgG2b CD3 ***mAb*** and a monovalent hybrid ***rat*** IgG2b- ***mouse*** IgG1 ***bispecific*** Ab triggered T cell activation in PBMC. Inhibition analyses with ***mAb*** blocking different human IgG Fc receptors (Fc gamma R) showed a dimorphic pattern. In donors expressing an Fc gamma RIIa-R/R131 allotype (previously defined on the basis of interaction with ***mouse*** (m) IgG1 as "high responder") anti-Fc gamma RI ***mAb*** 197 inhibited ***rat*** IgG2b induced T cell mitogenesis almost completely. In Fc gamma RIIa-H/H131 ("low responder" allotype) donors, however, both anti-Fc gamma RI ***mAb*** 197 and anti-Fc gamma RII ***mAb*** IV.3 were essential for optimal inhibition of mitogenesis. T cell proliferation experiments performed with the use of Fc gamma R-transfected fibroblasts as accessory cells showed the high affinity Fc gamma RIa (CD64) to interact with both ***rat*** IgG2b and ***rat*** IgG2b-mIgG1 hybrid CD3 ***mAb*** . The use of the two types of Fc gamma RIIa (CD32)-transfectants instead showed ***rat*** IgG2b CD3 ***mAb*** to interact solely with the IIa-H/H131 allotype. Interestingly, ***rat*** IgG2b-mIgG1 hybrid ***mAb*** did not interact effectively with this low affinity Fc gamma R. This suggests a requirement for only

one ***rat*** IgG2b H chain for Fc gamma RIa-mediated binding, whereas two identical H chains seem to be necessary for proper interaction with Fc gamma RIIa. Ab-sensitized RBC-rosette experiments performed with the use of a ***rat*** IgG2b anti-NIP ***mAb*** confirmed the interaction pattern observed with ***rat*** CD3 ***mAb*** , supporting the phenomena to be isotype-, and not ***mAb*** -, dependent. These analyses point to a unique reactivity pattern for ***rat*** IgG2b Abs, interacting both with the high affinity Fc gamma RIa in all donors and Fc gamma RIIa of individuals expressing the IIa-H131 allotype.

L6 ANSWER 22 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:395777 BIOSIS
DOCUMENT NUMBER: PREV199598410077
TITLE: CD8 T cell activation after intravenous administration of CD3 X CD19 ***bispecific*** ***antibody*** in patients with non-Hodgkin lymphoma.
AUTHOR(S): De Gast, Gijsbert C. (1); Haagen, Inez-Anne; Van Houten, Anja A.; Klein, Sigrid C.; Duits, Ashley J.; De Weger, Roel A.; Vroom, Thea M.; Clark, Mike R.; Phillips, Jenny; Van Dijk, Anette J. G.; De Lau, Wim B. M.; Bast, Bert J. E. G.
CORPORATE SOURCE: (1) Dep. Haematol., Univ. Hosp. Utrecht, P.O. Box 85500, 3508 GA Utrecht Netherlands
SOURCE: Cancer Immunology Immunotherapy, (1995) Vol. 40, No. 6, pp. 390-396.
ISSN: 0340-7004.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A ***bispecific*** ***antibody*** directed to T and B cells (CD3 times CD19 bsAb) was daily infused intravenously in escalating doses from 10 mu-g up to 5 mg in three patients with chemotherapy-resistant non-Hodgkin lymphoma; in this way we aimed to activate T cells to kill the malignant B cells. Only limited toxicity was observed, consisting of moderate fever preceded by chills or shivers and mild thrombocytopenia. No human anti( ***mouse*** Ig) antibodies were found. Pharmacokinetics showed a t-1/2 of 10.5 h with peak levels of 200-300 ng/ml after infusion of 2.5 mg bsAb. bsAb in serum was functionally active in vitro. After bsAb infusion a rise in serum tumour necrosis factor alpha was observed, accompanied by an increase in soluble CD8 and to some extent in soluble interleukin-2 receptor (IL-2R), but not in interferon gamma, IL-4 or soluble CD4. No evidence was found for monocyte activation (no increases in IL-6, IL-8 or IL-1-beta in serum). No gross changes in histology or number of IL-2R+, CD4+ or CD8+ cells were found in the lymph nodes after therapy, but one patient showed activated CD8+ T cells within the tumour nodules. In conclusion, after intravenously administered CD3 times CD19 bsAb only moderate toxicity was found, probably due to CD8+ T cell activation and cytokine release, without CD4+ T cell activation.

L6 ANSWER 23 OF 49 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 95325592 MEDLINE
DOCUMENT NUMBER: 95325592 PubMed ID: 7602098
TITLE: Preferential species-restricted heavy/light chain pairing in ***rat*** / ***mouse*** quadromas. Implications for a single-step purification of ***bispecific*** antibodies.
AUTHOR: Lindhofer H; Mocikat R; Steipe B; Thierfelder S
CORPORATE SOURCE: GSF, Immunology Institute, Munich, Germany.
SOURCE: JOURNAL OF IMMUNOLOGY, (1995 Jul 1) 155 (1) 219-25.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Space Life Sciences
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950822
Last Updated on STN: 19950822
Entered Medline: 19950807

AB Conventional ***mouse*** / ***mouse*** or ***rat*** / ***rat*** hybrid-hybridoma supernatants contain up to 10 different IgG molecules consisting of various combinations of heavy and light chains. Hence, the yield of functional ***bispecific*** Ab is low, and purification is often complicated, hampering a general preclinical evaluation of, e.g., ***bispecific*** Ab-mediated tumor immunotherapy in animal models. In experiments to overcome this drawback we found that fusion of ***rat*** with ***mouse*** hybridomas opens the possibility of large scale production of ***bispecific*** Ab due to the increased incidence of correctly paired Ab and facilitated purification. In essence, ***rat*** / ***mouse*** ***quadroma*** -derived ***bispecific*** Ab have the following advantages: 1) enrichment of functional ***bispecific*** Ab because of preferential species-restricted heavy/light chain pairing (observed in four of four ***rat*** - ***mouse*** quadromas) in contrast to the random pairing in conventional ***mouse*** / ***mouse*** or ***rat*** / ***rat*** quadromas, and 2) a possible one-step purification of the ***quadroma*** supernatant with protein A. This simple chromatography step does not bind unwanted variants with parental ***rat*** / ***rat*** heavy chain configuration, and the desired ***rat*** / ***mouse*** ***bispecific*** Ab are retained, which can then easily be separated from parental ***mouse*** Ab by sequential pH elution.

L6 ANSWER 24 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:362606 BIOSIS
DOCUMENT NUMBER: PREV199598376906
TITLE: Preferential species-restricted heavy/light chain pairing in ***rat*** / ***mouse*** quadromas: Implications for a single-step purification of ***bispecific*** antibodies.
AUTHOR(S): Lindhofer, Horst; Mocikat, Ralph; Steipe, Boris; Thierfelder, Stefan (1)
CORPORATE SOURCE: (1) GSF-Inst. Immunologie, Marchioninistr. 25, 81377 Munich Germany
SOURCE: Journal of Immunology, (1995) Vol. 155, No. 1, pp. 218-225. ISSN: 0022-1767.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Conventional ***mouse*** / ***mouse*** or ***rat*** / ***rat*** hybrid-hybridoma supernatants contain up to 10 different IgG molecules consisting of various combinations of heavy and light chains. Hence, the yield of functional ***bispecific*** Ab is low, and purification is often complicated, hampering a general preclinical evaluation of, e.g., ***bispecific*** Ab-mediated tumor immunotherapy in animal models. In experiments to overcome this drawback we found that fusion of ***rat*** with ***mouse*** hybridomas opens the possibility of large scale production of ***bispecific*** Ab due to the increased incidence of correctly paired Ab and facilitated purification. In essence, ***rat*** / ***mouse*** ***quadroma*** -derived ***bispecific*** Ab have the following advantages: 1) enrichment of functional ***bispecific*** Ab because of preferential species-restricted heavy/light chain pairing (observed in four of four ***rat*** - ***mouse*** quadromas) in contrast to the random pairing in conventional ***mouse*** / ***mouse*** or ***rat*** / ***rat*** quadromas, and 2) a possible one-step purification of the ***quadroma*** supernatant with protein A. This simple chromatography step does not bind unwanted variants with parental ***rat*** / ***rat*** heavy chain configuration, and the desired ***rat*** / ***mouse*** ***bispecific*** Ab are retained, which can then easily be separated from parental ***mouse*** Ab by sequential pH elution.

L6 ANSWER 25 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 95061782 EMBASE
DOCUMENT NUMBER: 1995061782
TITLE: Multistep tumor targeting in nude mice using ***bispecific*** antibodies and a gallium chelate suitable for immunoscintigraphy with positron emission

tomography.
AUTHOR: Schuhmacher J.; Klivenyi G.; Matys R.; Stadler M.; Regiert T.; Hauser H.; Doll J.; Maier-Borst W.; Zoller M.
CORPORATE SOURCE: Diagnostic/Therapeutic Radiol. Dept., German Cancer Research Center, Im Neuenheimer Feld 280,69009 Heidelberg, Germany
SOURCE: Cancer Research, (1995) 55/1 (115-123).
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
023 Nuclear Medicine
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB To improve tumor:tissue ratios in immunoscintigraphy, a three-step targeting method has been developed. The reagents used were (a) a radioactive, low molecular weight chelate prepared from ionic gallium and a phenolic polyaminocarboxylic acid, which can be labeled either with the single-photon emitter 67Ga or with the short-lived positron emitter 68Ga (t1/2 = 68 min); (b) a ***bispecific*** monoclonal ***antibody*** (bs- ***mAb*** ) synthesized from the F(ab)2 fragment of the 1.1ASML ***antibody*** specific for the glycoprotein CD44v associated with a ***rat*** pancreas carcinoma cell line and the F(ab') fragment of an ***antibody*** specific for the gallium chelate; and (c) the nonradioactive gallium chelate covalently coupled to transferrin, which served as a high molecular weight blocker to prevent binding of the radioactive gallium chelate to bs-mAbs in the circulation. Targeting experiments in tumor-bearing nude mice with different doses of bs-mAbs, blocker, and 67Ga chelate were adjusted to maximize tumor to tissue contrasts and tumor uptake. Compared with the biodistribution of the 131I-labeled, native 1.1ASML ***antibody*** 24 h postinjection, a schedule using 100 pmol bs- ***mab*** 24 h later 100 pmol blocker, 15 min later 16 pmol 67Ga chelate, 1 h later examination, increased tumor:blood and tumor: liver ratios by a factor of 5 while keeping the localization of radioactivity in the tumor constant (10.1% injected dose/g). High-contrast images using either 67Ga or 68Ga were obtained within 1 h. The targeting method described enables the use of the short-lived positron emitter 68Ga and thus allows the combination of an improved immunoscintigraphy and positron emission tomography.

L6 ANSWER 26 OF 49 MEDLINE on STN
ACCESSION NUMBER: 95249177 MEDLINE
DOCUMENT NUMBER: 95249177 PubMed ID: 7731620
TITLE: Immunotechnological trends in radioimmunotargeting: from 'magic bullet' to 'smart bomb'.
AUTHOR: Hazra D K; Britton K E; Lahiri V L; Gupta A K; Khanna P; Saran S
CORPORATE SOURCE: Postgraduate Department of Medicine, S.N. Medical College, Agra, India.
SOURCE: NUCLEAR MEDICINE COMMUNICATIONS, (1995 Feb) 16 (2) 66-75.
Ref: 22
Journal code: 8201017. ISSN: 0143-3636.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 19950608
Entered Medline: 19950530
AB The impact of recent advances in the chemical and genetic engineering manipulations of antibodies on radioimmunotargeting is reviewed both in relation to radioimmunoscintigraphy and radioimmunotherapy. The resulting trends are: (1) the linking of parts of the ***mouse*** / ***rat***

and human ***antibody*** molecule; (2) the creation of molecules with dual antigen or multiple antigen recognition capabilities; (3) the making of smaller and smaller antigen recognition molecules; and (4) the development of molecules with dual capabilities, e.g. antigen recognition and enzyme activity. The various methods of creating antibodies in vitro are reviewed with reference to bacteria, using phage selection and a combinatorial library, mammalian cells, yeast cells and, finally, mice containing giant yeast artificial chromosomes. The advantages and disadvantages of smaller fragments as well as of the human anti- ***mouse*** ***antibody*** (HAMA) reaction are discussed and the need for early clinical evaluation and widespread availability of the newer antibodies is emphasized. It is envisaged that these immunotechnological advances will permit the large-scale production of precisely engineered humanized antibodies, and the specificity and affinity rate constant of these antibodies can be optimized using in vitro phage selection as well as by computer modelling where the stereo chemistry of the antigen is known precisely.

L6 ANSWER 27 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 94156812 EMBASE
DOCUMENT NUMBER: 1994156812
TITLE: Efficient tumor cell lysis mediated by a ***bispecific*** single chain ***antibody*** expressed in Escherichia coli.
AUTHOR: Gruber M.; Schodin B.A.; Wilson E.R.; Kranz D.M.
CORPORATE SOURCE: Department of Biochemistry, University of Illinois,Urbana, IL 61801, United States
SOURCE: Journal of Immunology, (1994) 152/11 (5368-5374).
ISSN: 0022-1767 CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Recent advances in the expression of Abs in Escherichia coli have raised the possibility that virtually any specificity can be obtained by either cloning Ab genes from characterized hybridomas or by de novo selection using Ab gene libraries. ***Bispecific*** Abs have been more difficult to engineer because of problems inherent in the proper folding and association of V(H) and V(L) domains. In this report, a model system for expressing and testing the activity of a single chain ***bispecific*** Ab was used. The Ab contained the V(H) and V(L) genes from the anti-TCR Ab 1B2 joined by a 25 amino acid residue linker to the V(H) and V(L) genes from the anti-fluorescein Ab 4420. The 57-kDa single chain ***bispecific*** Ab (scF(V2)) was purified in a single step by affinity chromatography through a fluorescein column at a yield of 1 mg/L of bacterial culture. Despite the presence of 1B2 V regions at the NH2-terminus and a 10-residue c-myc peptide at the COOH-terminus, the refolded protein had an affinity for fluorescein that was nearly identical with the monospecific single chain Ab. The scF(V2) also bound the TCR of the ***mouse*** CTL clone 2C and redirected the lysis of human tumor cells that had fluorescein covalently linked to their surface. Lysis was mediated at scF(V2) concentrations that were 100-fold lower than the concentrations of Ab that inhibited normal recognition by CTL 2C. These results show that single chain ***bispecific*** Abs can mediate CTL lysis of target cells without the immunosuppressive side effects associated with the use of anti-TCR Abs.

L6 ANSWER 28 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 94148413 EMBASE
DOCUMENT NUMBER: 1994148413
TITLE: Induction of a protective human polysaccharide-specific ***antibody*** response in hu-PBL SCID mice by idiotypic vaccination.

AUTHOR: Reason D.C.; Kitamura M.Y.; Lucas A.H.
CORPORATE SOURCE: Children's Hosp. Oakland Res. Inst.,Oakland, CA 94609, United States
SOURCE: Journal of Immunology, (1994) 152/10 (5009-5013).
ISSN: 0022-1767 CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The human Ab repertoire to the Haemophilus influenzae type b (Hib) polysaccharide (PS) is dominated by Abs that use the .kappa.II-A2 V(L) region and that express an idiotype (Id) designated Hibld-1. In this study we determined whether a human Hib PS-specific Ab response could be induced by idiotypic manipulation. We prepared a ***bispecific*** vaccine consisting of the F(ab')2 fragment of a ***mAb*** specific for Hibld-1, coupled to the F(ab')2 fragment of a ***mAb*** specific for CD3, a component of the TCR complex. This ***bispecific*** idiotypic vaccine stimulated production of human Abs to Hib PS in severe combined immunodeficient mice engrafted with normal human adult PBLs. The induced Abs uniformly expressed Hibld-1 and protected neonatal rats from Hib bacteremia. Experiments using additional conjugates demonstrated that covalent coupling of the CD3-specific moiety to the anti-Id was required for immunogenicity in this model, a result suggesting that engagement of B cell Id and proximate delivery of T cell signals are both necessary for B cell activation and differentiation. These findings demonstrate that human Ids can serve as targets for induction of a protective anti-PS Ab response.

L6 ANSWER 29 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:468541 BIOSIS
DOCUMENT NUMBER: PREV199497481541
TITLE: ***Rat*** - ***mouse*** quadromas allow augmented generation of ***bispecific*** antibodies and single step purification: First in vivo studies.
AUTHOR(S): Lindhofer, H.; Menzel, H.; Thierfelder, S.
CORPORATE SOURCE: GSF-Inst. Immunologie, Munich Germany
SOURCE: Experimental Hematology (Charlottesville), (1994) Vol. 22, No. 8, pp. 763.
Meeting Info.: 23rd Annual Meeting of the International Society for Experimental Hematology Minneapolis, Minnesota, USA August 21-25, 1994
ISSN: 0301-472X.
DOCUMENT TYPE: Conference
LANGUAGE: English

L6 ANSWER 30 OF 49 MEDLINE on STN
ACCESSION NUMBER: 94327196 MEDLINE
DOCUMENT NUMBER: 94327196 PubMed ID: 8050776
TITLE: Production and in vivo characterization of a bifunctional ***antibody*** (IVA039.1) with specificity for the ***mouse*** interleukin-2 receptor and vinca alkaloids.
AUTHOR: Kuus-Reichel K; Knott C L; Sam-Fong P; Jue R A; Mackensen D G; Corvalan J R
CORPORATE SOURCE: Hybritech Incorporated, San Diego CA 92196-9006.
SOURCE: HYBRIDOMA, (1994 Apr) 13 (2) 115-22.
Journal code: 8202424. ISSN: 0272-457X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19940914
Last Updated on STN: 19940914
Entered Medline: 19940906
AB The autoreactive T cell plays a pivotal role in the pathogenesis of type I

diabetes in humans and in rodent animal models. Elimination or attenuation of these cells may provide a means to treat the disease. The use of antibodies directed to T cells has shown varying degrees of effectiveness in the treatment of autoimmune disease. The use of a bifunctional ***antibody*** directed to T cells with a cytolytic agent may provide an additional level of therapeutic efficacy compared to anti-T-cell antibodies alone. To test this hypothesis, we prepared a bifunctional ***antibody*** (IVA039.1) with specificity for the ***mouse*** interleukin-2 (IL-2) receptor and vinca alkaloids. The ***antibody*** was derived from the fusion of vinca immune spleen cells with PC61 5.3, a hybridoma that produces ***rat*** anti- ***mouse*** IL-2 receptor ***antibody*** . IVA039.1 was purified by affinity chromatography through Protein A and anti-vinca affinity columns followed by TSK-DEAE high-pressure liquid chromatography (HPLC). Bifunctionality of the ***antibody*** was confirmed by fluorescence-activated cell sorting (FACS) analysis, enzyme-linked immunoadsorbent assay (ELISA) and a cell assay designed to measure simultaneously both IL-2 receptor and vinca reactivities. The biodistribution of IVA039.1 was determined in normal and streptozotocin-complete Freund's adjuvant (CFA) induced diabetic mice. Enhanced uptake of IVA039.1 was observed in the pancreata, spleens, and lymph nodes of diabetic compared to normal mice. These data suggest that bifunctional antibodies that can deliver cytolytic agents to T cells may be appropriate candidates for the treatment of diabetes and other autoimmune diseases.

L6 ANSWER 31 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1993:656537 CAPLUS
DOCUMENT NUMBER: 119:256537
TITLE: Diagnostic and/or therapeutic immunoconjugates targeted to neovascular endothelial cells
INVENTOR(S): Thorpe, Philip E.; Burrows, Francis J.
PATENT ASSIGNEE(S): University of Texas System, USA; Imperial Cancer Research Technology
SOURCE: PCT Int. Appl., 171 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 9
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9317715 | A1 | 19930916 | WO 1993-US1956 | 19930305 |
| W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG | | | | |
| AU 9337378 | A1 | 19931005 | AU 1993-37378 | 19930305 |
| EP 627940 | A1 | 19941214 | EP 1993-906289 | 19930305 |
| EP 627940 | B1 | 20030507 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| EP 1306095 | A2 | 20030502 | EP 2002-24529 | 19930305 |
| EP 1306095 | A3 | 20030625 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE | | | | |
| AT 239506 | E | 20030515 | AT 1993-906289 | 19930305 |
| US 6004554 | A | 19991221 | US 1994-295868 | 19941202 |

PRIORITY APPLN. INFO.: US 1992-846349 A2 19920305
EP 1993-906289 A3 19930305
WO 1993-US1956 A 19930305

AB An ***antibody*** or ***antibody*** fragment that recognizes a cell surface antigen assocd. with endothelial vasculature of a vascularized tumor mass is linked to a therapeutic or diagnostic agent for treatment or diagnosis of vascularized tumors. The ***antibody*** may be linked to a paramagnetic or radioactive ion, cytotoxic agent, cytokine, etc. Thus, a neuroblastoma transfected with the ***mouse*** .gamma.-interferon gene was grown in mice with severe combined

immunodeficiency. The .gamma.-interferon secreted by the tumor induced expression of MHC class II antigens on the tumor vascular endothelium. A ***rat*** IgG2b monoclonal ***antibody*** which recognized MHC Ia antigens, conjugated to deglycosylated ricin A chain, was used successfully for treatment of the neuroblastoma.

L6 ANSWER 32 OF 49 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 93246675 MEDLINE
DOCUMENT NUMBER: 93246675 PubMed ID: 8482850
TITLE: Construction of a ***bispecific*** ***antibody*** reacting with the alpha- and beta-chains of the human IL-2 receptor. High affinity cross-linking and high anti-proliferative efficiency.
AUTHOR: Francois C; Boeffard F; Kaluza B; Weidle U H; Jacques Y
CORPORATE SOURCE: Institut National de la Sante et de la Recherche Medicale (INSERM U211), Nantes, France.
SOURCE: JOURNAL OF IMMUNOLOGY, (1993 May 15) 150 (10) 4610-9. Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930618
Last Updated on STN: 19930618
Entered Medline: 19930603
AB A ***bispecific*** ***antibody*** recognizing both the alpha- and beta-chains of the IL-2R was generated by sulfhydryl-directed chemical reassociation of monovalent Fab' fragments prepared from the anti-alpha ***mAb*** 33B3.1 ( ***rat*** IgG2a) and from the anti-beta ***mAb*** A41 ( ***mouse*** IgG1). Whereas the 33B3.1/A41 ***bispecific*** ***mAb*** (bi- ***mAb*** ) binds to isolated alpha- and beta-chains with low affinity (Kd = 4 nM), its binding to cells co-expressing the two chains shows both low and high affinity components. The high affinity-binding sites (Kd = 100 pM) most probably correspond to the cross-linking by the bi- ***mAb*** of alpha- and beta-chains, whereas the low affinity component corresponds to the excess of alpha-chains. High affinity binding of bi- ***mAb*** on activated T cells is observed at 37 degrees C and not at 4 degrees C, suggesting that i) the two chains are dissociated at 4 degrees C in the absence of ligand and ii) the mechanism of bi- ***mAb*** catalyzed cross-linking of these two chains is temperature dependent. In contrast to parental 33B3.1 and A41 IgG, which recognize single positive (alpha + and beta +, respectively) and double positive alpha +/beta + cells with similar affinities, the 33B3.1/A41 bi- ***mAb*** is specific for activated alpha +/beta + cells with respect to its high affinity binding. In contrast to A41, which does not affect IL-2-induced proliferation of 4AS cells or anti-CD3-activated PBL, and to 33B3.1, which do inhibit proliferation but only partially and at high doses, the bi- ***mAb*** showed full blocking efficiencies at low concentrations (IC50 of 300 to 400pM) corresponding to the formation of high affinity alpha/bi- ***mAb*** /beta complexes. These half-maximal effects were observed at 10-fold lower concentrations than when using a combination of equimolar concentrations of parental 33B3.1 and A41 IgG. Because of their specificity and high blocking efficiencies, anti-alpha/anti-beta bi- ***mAb*** may constitute a better alternative for IL-2R-directed immunosuppression.

L6 ANSWER 33 OF 49 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 93272249 MEDLINE
DOCUMENT NUMBER: 93272249 PubMed ID: 8500112
TITLE: The development and purification of a ***bispecific*** ***antibody*** for lymphokine-activated killer cell targeting against the ***rat*** colon carcinoma CC531.
AUTHOR: Kuppen P J; Eggermont A M; Smits K M; van Eendenburg J D; Lazeroms S P; van de Velde C J; Fleuren G J
CORPORATE SOURCE: Department of Pathology, University of Leiden, The

Netherlands.
SOURCE: CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1993 Jun) 36 (6) 403-8.
Journal code: 8605732. ISSN: 0340-7004.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930716
Last Updated on STN: 19970203
Entered Medline: 19930630

AB In vivo targeting of lymphokine-activated killer (LAK) cells to tumour deposits by ***bispecific*** monoclonal antibodies (bimAb) may be a way to improve adoptive immunotherapy. We developed a bimAb against adherent LAK (ALAK) cells and colon tumour CC531 in Wag rats. The bimAb was produced by somatic hybridization of two ***mouse*** hybridomas, one producing monoclonal antibodies ( ***mAb*** ) against CD8 (IgG2b, OX8), and the other producing ***mAb*** against a CC531-associated antigen (IgG1, CC52). A bimAb-producing clone was selected by an enzyme-linked immunosorbent assay with CC531 tumour cells. BimAb were purified from ascitic fluid by protein A affinity chromatography. Each of five pooled peak fractions was analysed by flow cytometry for the presence of bimAb. Most bimAb were found in a fraction that was eluted at pH 4.5 from protein A. FPLC analysis of this fraction revealed that no parental antibodies were present. The OX8 x CC52 bimAb greatly increased conjugate formation in vitro between ALAK cells and CC531. Results of 51Cr-release assays with CC531 as target cells and ALAK cells as effector cells were not significantly different in the presence or in the absence of the bimAb. The methods we used here, a cell enzyme-linked immunosorbent assay and flow cytometry, are simple methods for development and purification of a bimAb when a functional selection method is not a priori available. The OX8 x CC52 bimAb we developed this way may increase in vivo tumour targeting of ALAK cells and thus augment antitumour effect in vivo.

L6 ANSWER 34 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1993:344953 BIOSIS
DOCUMENT NUMBER: PREV199396041953
TITLE: T-cell retargeting using ***bispecific*** monoclonal antibodies in a ***rat*** colon carcinoma model: III. Activation of resting T cells and tumor neutralization induced by ***bispecific*** antibodies.
AUTHOR(S): Beun, Gideon D. M. (1); Van De Velde, Cornelis J. H.; Fleuren, Gert Jan
CORPORATE SOURCE: (1) Dep. Hematol., Dr. Daniel den Hoed Cancer Center, Groene Hilledijk 301, PO Box 5201, 3008 AE Rotterdam Netherlands Antilles
SOURCE: Journal of Immunotherapy, (1993) Vol. 13, No. 4, pp. 223-231.
ISSN: 1053-8550.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We investigated the ability of two murine ***bispecific*** anti-***rat*** T-cell receptor times anti-tumor antibodies, composed of dual IgG-1 or IgG-1 times IgG-2b isotypes, to activate resting T lymphocytes in fresh, unfractionated ***rat*** spleen cell populations. The dual IgG-1 ***antibody*** was found to be a potent activator, whereas the IgG-1 times IgG-2b ***antibody*** was considerably less active. However, on prolonged cocultivation of spleen cells and syngeneic CC531 colon tumor cells, both antibodies induced spleen cell proliferation and tumor neutralization if exogenous IL-2 was present. Their functional activities suggest that these bi-specific antibodies should be able, upon in vivo administration, to recruit endogenous T lymphocytes as activated, cytotoxic effector cells. Exploitation of these biological characteristics may be incorporated in the design of therapeutic trials in this model.

L6 ANSWER 35 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 93023865 EMBASE

DOCUMENT NUMBER: 1993023865
TITLE: ***Bispecific*** ***antibody*** therapy.
AUTHOR: Brissinck J.; Demanet C.; Leo O.; Thielemans K.
CORPORATE SOURCE: Hematology-Immunology, Medical School, Vrije Universiteit, Laarbeeklaan 103/E,1090 Brussels, Belgium
SOURCE: Drugs of the Future, (1992) 17/11 (1003-1010).
ISSN: 0377-8282 CODEN: DRFUD4
COUNTRY: Spain
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
016 Cancer
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English

L6 ANSWER 36 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:654148 CAPLUS
DOCUMENT NUMBER: 115:254148
TITLE: Methods and compositions for promoting immunopotentiation
INVENTOR(S): Bluestone, Jeffery A.
PATENT ASSIGNEE(S): Arch Development Corp., USA
SOURCE: PCT Int. Appl., 112 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9106319 | A1 | 19910516 | WO 1990-US6177 | 19901026 |
| W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU | | | | |
| RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG | | | | |
| CA 2071478 | AA | 19910428 | CA 1990-2071478 | 19901026 |
| AU 9066423 | A1 | 19910531 | AU 1990-66423 | 19901026 |
| EP 497883 | A1 | 19920812 | EP 1990-916853 | 19901026 |
| EP 497883 | B1 | 19980715 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP 05504554 | T2 | 19930715 | JP 1990-515665 | 19901026 |
| JP 2546544 | B2 | 19961023 | | |
| EP 839536 | A1 | 19980506 | EP 1998-100138 | 19901026 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT 168272 | E | 19980815 | AT 1990-916853 | 19901026 |
| US 6113901 | A | 20000905 | US 1994-286805 | 19940805 |
| US 6143297 | A | 20001107 | US 1995-458462 | 19950602 |
| US 6406696 | B1 | 20020618 | US 1995-459486 | 19950602 |

PRIORITY APPLN. INFO.: US 1989-429729 A 19891027
US 1990-524304 A 19900516
EP 1990-916853 A3 19901026
WO 1990-US6177 A 19901026
US 1992-990553 B1 19921214
US 1994-286805 A3 19940805

AB This invention discloses immunopotentiating agents which stimulate an immune response. These agents are single agents that act directly, adjuvants added concurrently with the agents, or heteroconjugates. Heteroconjugate agents elicit or enhance a cellular or humoral immune response which may be specific for an epitope contained within an amino acid sequence. Enhanced hematopoieses by bone marrow stem cell recruitment was also a result of administering some of these agents. Examples of immunopotentiating agents include monoclonal antibodies and proteins derived from microorganisms (e.g., enterotoxins) which activate T-cells. One method of treatment disclosed uses only the immunopotentiating agent to stimulate the immune system. Another uses

adjuvants in combination with the agent. A third method employs heteroconjugates comprising (a) an immunopotentiating protein which is characterized as having an ability to stimulate T-cells; and (b) a second protein having an amino acid sequence which includes an epitope against which a cellular or humoral response is desired. This invention also relates to a method of prepg. the heteroconjugate, and to a method of stimulating the immune system in vivo in a novel way. One route of stimulation is to activate T-cells, in some instances, specific subsets of T-cells, by administering heteroconjugates contg. an immunopotentiating protein and a second protein, to mammals. For this method of treatment, the second protein in the heteroconjugate is derived from abnormal or diseased tissue, or from an infectious agent; alternatively, the second protein is produced synthetically by std. methods of mol. biol. Sources of the second protein include tumors, viruses, bacteria, fungi, protozoal or metozoal parasites. Monoclonal antibodies or T-cells prepd. from mammals whose immune systems have responsed to administration of the heteroconjugate may be produced and administered to induce passive immunity. A method of prepg. a hybridoma which secretes the monoclonal antibodies and use of these monoclonal antibodies and T-cells, are also disclosed. This invention is also directed to a vaccine comprising the heteroconjugate. Administration of low doses of monoclonal anti-CD3 prevented lethal pneumonia caused by Sendai virus in >60% of mice. Anti-CD3-treated, virally-infected mice also developed lasting virus-specific immunity. The 129/J strain of mice was also protected.

L6 ANSWER 37 OF 49 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 92239837 MEDLINE
DOCUMENT NUMBER: 92239837 PubMed ID: 1687361
TITLE: [A new approach to the design of hybrid hybridomas based on the use of an actinomycin D-resistant line of murine myeloma].
Novyi podkhod k konstruirovaniiu gibridnykh gibridom, osnovannyi na ispol'zovanii aktinomitsin-D-rezistentnoi linii mielomy myshi.
AUTHOR: Massino Iu S; Kizim E A; Dmitriev A D
SOURCE: BIULLETEN EKSPERIMENTALNOI BIOLOGII I MEDITSINY, (1991 Nov) 112 (11) 511-4.
Journal code: 0370627. ISSN: 0365-9615.
PUB. COUNTRY: USSR
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19950206
Entered Medline: 19920529
AB The hybrid hybridomas (tetradomas) were produced from the fusion of the double mutant actinomycin Dr (ADr)/HATs hybridoma to horseradish peroxidase (HRP) and wild type hybridoma to alpha-endorphin (EP). The double mutant phenotype was constructed using the new strategy, based on the fusion of immune ***mouse*** splenocytes with ***mouse*** myeloma (X63.Ag8, 653) cell variants, made resistant to 30 ng/ml of AD by stepwise selection. This allowed the direct introduction of the dominant selective marker (ADr) into the hybrid cells. Tetradomas secreted the ***bispecific*** monoclonal antibodies (bi Mabs), simultaneously binding to EP and HRP in double antigen ELISA, the ELISA plates covered with EP-bovine serum albumin conjugate. Using ***rat*** pituitary the bi Mabs were shown to be effective for immunostaining of EP-producing cells. EP-producing cells.

L6 ANSWER 38 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:205190 CAPLUS
DOCUMENT NUMBER: 114:205190
TITLE: Two distinct monoclonal antibodies raised against ***mouse*** .beta. nerve growth factor. Generation of bi-specific anti-nerve growth factor anti-horseradish peroxidase antibodies for use in a

homogeneous enzyme immunoassay
AUTHOR(S): Kenigsberg, Rhoda L.; Elliott, Peter J.; Cuello, A. Claudio
CORPORATE SOURCE: Dep. Pharm. Ther., McGill Univ., Montreal, QC, H3G 1Y6, Can.
SOURCE: Journal of Immunological Methods (1991), 136(2), 247-57
CODEN: JIMMBG; ISSN: 0022-1759
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two hybridomas producing monoclonal antibodies against ***mouse*** .beta. nerve growth factor (NGF) were obtained from the fusion of hyperimmune splenocytes from rats immunized with polymd. .beta.-NGF and Sp2/0.Ag ***mouse*** myeloma cells. The monoclonal antibodies coded IgG 24 and 30 produced and secreted by the hybrid cells are both of the IgG2a subclass. Both monoclonal antibodies are capable of recognizing native NGF coated on microassay plates as well as the denatured factor on Western blots. However, only IgG 30 could block NGF-induced process outgrowth from the ***rat*** pheochromocytoma cell line (PC12) as well as NGF-induced increase in choline acetyltransferase activity in ***rat*** primary septal cell cultures. In addn., only IgG 30 could detect immunocytochem. NGF-immunoreactive sites in fixed tissue. And, finally, IgG 24 could not compete for IgG 30 binding to immobilized native NGF. Consequently, it appears that these antibodies are recognizing different epitopes on the NGF mol. Neither monoclonal ***antibody*** displayed any crossreactivity with serum albumin, aprotinin, epidermal growth factor, or insulin. A hybrid-hybridoma producing bi-specific anti-NGF anti-horseradish peroxidase (HRP) monoclonal antibodies was generated from the fusion of an azaguanine resistant anti-HRP hybridoma, coded RAP2.Ag and the anti-NGF IgG 30 hybridoma treated with emetine. The potential merits of using these bi-specific antibodies in combination with their mono-specific anti-NGF parent in a homogeneous sandwich immunoassay for the quantitation of NGF are discussed.

L6 ANSWER 39 OF 49 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 90370047 MEDLINE
DOCUMENT NUMBER: 90370047 PubMed ID: 1697645
TITLE: Purification and analysis of ***bispecific*** tetrameric ***antibody*** complexes.
AUTHOR: Lansdorp P M; Thomas T E
CORPORATE SOURCE: Terry Fox Laboratory, Cancer Control Agency, Vancouver, British Columbia, Canada.
SOURCE: MOLECULAR IMMUNOLOGY, (1990 Jul) 27 (7) 659-66. Journal code: 7905289. ISSN: 0161-5890.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901109
Last Updated on STN: 19960129
Entered Medline: 19901011
AB In order to study the type and yield of immune complexes obtained by the mixing of purified F(ab')2 fragments of ***rat*** monoclonal antibodies specific for ***mouse*** IgG1 with equimolar amounts of purified ***mouse*** IgG1 size exclusion HPLC of the reaction mixture was performed. Immune complexes eluted as a single peak at a position compatible with a tetrameric ***antibody*** complex configuration. The yield of tetramers could be increased by incubation of the ***antibody*** mixture for several hours at 37 degrees C, indicating a preference of the tetrameric composition over other immune complex compositions. Size exclusion HPLC also showed that greater than 80% of purified tetramers retained their original dimensions after storage for 1 year at 4 degrees C, thus indicating the long-term stability of tetrameric ***antibody*** complexes. When complexes were prepared with a mixture of two different ***mouse*** IgG1 antibodies, ***bispecific*** tetramers were obtained that could be separated from monospecific

tetramers using DEAE-HPLC. Purified ***bispecific*** ***antibody*** complexes of ***mouse*** IgG1 anti-CD34 (My10) cross-linked to ***mouse*** IgG1 anti-desferal with F(ab')2 ***rat*** anti-***mouse*** IgG1 were useful for the purification of cells expressing CD34 from human bone marrow. For this purpose cells were labelled with the ***antibody*** complexes, selectively adsorbed onto columns containing desferal coated glass beads and then selectively eluted by treatment with dithiothreitol resulting in reductive cleavage of the disulfide bonds of the F(ab')2 fragments. This relatively simple cell fractionation technique illustrates the unique cross-linking properties of ***bispecific*** tetrameric ***antibody*** complexes. The procedure appears useful for further studies of hemopoietic cells and bone marrow transplantation.

L6 ANSWER 40 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
DUPLICATE 11
ACCESSION NUMBER: 90142250 EMBASE
DOCUMENT NUMBER: 1990142250
TITLE: The simpliRED D dimer test: A novel assay for the detection of crosslinked fibrin degradation products in whole blood.
AUTHOR: John M.A.; Elms M.J.; O'Reilly E.J.; Rylatt D.B.; Bundesen P.G.; Hillyard C.J.
CORPORATE SOURCE: Agen Biomedical Limited, 11 Durbell Street, P.O. Box 391, Acacia Ridge, QLD 4110, Australia
SOURCE: Thrombosis Research, (1990) 58/3 (273-281).
ISSN: 0049-3848 CODEN: THBRAA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A new system for the detection of fibrin degradation products in whole blood has been developed. The test provides a clearly visible agglutination of the patient's red blood cells in the presence of elevated levels of the crosslinked fibrin derivative, D dimer. The test, which uses a ***bispecific*** reagent prepared from Fab' fragments of monoclonal antibodies, gives a positive result in 1-2 minutes. One monoclonal ***antibody*** ( ***RAT*** -1C3/86) was raised against human red blood cells, and the second (DD-3B6/22) was specific to the crosslinked fibrin derivative, D dimer. Addition of the ***bispecific*** reagent to a drop of patient's whole blood resulted in red blood cell agglutination when elevated levels of D dimers were present in the sample. Clinical trials showed sensitivity equivalent to that of current commercial tests. Samples from patients with thrombotic disease states as well as normals were examined. The test was compared with commercial latex agglutination and enzyme immunoassay systems and showed good correlation with the presence of elevated levels of crosslinked fibrin degradation products. This technology represents an advance which allows rapid 'on the spot' whole blood analysis, for the diagnosis of thrombotic disorders.

L6 ANSWER 41 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:154705 CAPLUS
DOCUMENT NUMBER: 112:154705
TITLE: Development of a bi-specific monoclonal ***antibody*** for simultaneous detection of rabbit IgG and horseradish peroxidase: use as a general reagent in immunocytochemistry and enzyme-linked immunosorbent assay
AUTHOR(S): Kenigsberg, Rhoda L.; Semenenko, Frances M.; Cuello, A. Claudio
CORPORATE SOURCE: Dep. Pharmacol. Ther., McGill Univ., Montreal, QC, Can.
SOURCE: Journal of Histochemistry and Cytochemistry (1990), 38(2), 191-8
CODEN: JHCYAS; ISSN: 0022-1554
DOCUMENT TYPE: Journal
LANGUAGE: English

AB ***Bispecific*** monoclonal antibodies ( ***MAb*** ) capable of simultaneous recognition of rabbit IgG and horseradish peroxidase (HRP) for use in a variety of immunobased techniques were developed. This ***bispecific*** ***antibody*** , McC8, was produced by fusion of the aminopterin-sensitive ***mouse*** hybridoma MAP.Ag.1, which secretes ***MAb*** against HRP and splenocytes from a ***mouse*** previously immunized with whole rabbit IgG. The resultant hybrid-hybridoma codominantly expresses and secretes the Ig chains, i.e., IgG1 and IgG2b, of its resp. parents, as detd. by radial immunodiffusion. The binding sites on rabbit IgG for McC8 were detd. on Western blots and in competition solid-phase enzymic immunoassays with the use of allotype-specific rabbit sera. Both these techniques demonstrated that McC8 recognizes the light chain of the rabbit IgG mol. with preferential binding to the B4 .kappa. light-chain allotype. McC8 was successfully used in 2-step immunocytochem. for localization of calcitonin gene-related peptide (CGRP) in fibers of the superfacial layers of the spinal trigeminal nucleus of the ***rat*** , as well as for localization of glial fibrillary acidic protein (GFAP)-immunoreactive sites in primary ***rat*** septal cell cultures, thus demonstrating its potential as a general developing reagent in conventional immunocytochem. McC8 compared favorably with peroxidase-antiperoxidase immunocytochem. with respect to sensitivity. However, the ***bispecific*** developing reagent proved superior to the conventional peroxidase-antiperoxidase procedure when both were employed in a similar fashion in tissues prone to display high background staining. Finally, McC8 was also employed as a developing reagent in a competitive ELISA designed for quantitation of CGRP with the use of a rabbit anti-CGRP primary ***antibody*** . The sensitivity of this quant. ELISA (190 pg or 50 fmol CGRP per well) renders this ***bispecific*** ***antibody*** suitable for use in quant. immunoassays for detection of relevant peptides in biol. systems.

L6 ANSWER 42 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:99590 CAPLUS
DOCUMENT NUMBER: 114:99590
TITLE: Production of a bi-specific monoclonal ***antibody*** recognizing ***mouse*** kappa light chains and horseradish peroxidase: applications in immunoassays
AUTHOR(S): Kenigsberg, R. L.; Cuello, A. C.
CORPORATE SOURCE: Dep. Pharmacol. Ther., McGill Univ., Montreal, QC, H3G 1Y6, Can.
SOURCE: Histochemistry (1990), 95(2), 155-63
CODEN: HCMYAL; ISSN: 0301-5564
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The prodn. of a bi-specific monoclonal ***antibody*** that simultaneously recognizes ***mouse*** kappa light chains and horseradish peroxidase (HRP) for use as a general developing reagent in a wide variety of immunobased techniques is described. This ***antibody*** , named McC10, was produced by the fusion of an aminopterin-sensitive interspecies hybridoma which secretes ***rat*** monoclonal antibodies against HRP (RAP2.cntdot.Ag) and splenocytes from a ***rat*** immunized with whole ***mouse*** IgG. The hybrid-hybridoma generated from this fusion expresses and secretes ***rat*** Igs of the IgG1 and IgG2a subclasses, as detd. by radial immunodiffusion. In competitive binding solid-phase enzymic assays, McC10 was found to cross-react with all four ***mouse*** IgG subclasses as well as ***mouse*** kappa light chains. In contrast, in this type of assay, McC10 did not appear to recognize ***mouse*** IgA, IgM or lambda light chains. However, IgM-bearing kappa light chains were recognized by immunocytochem. Epitope specificity of this bi-specific ***antibody*** was more clearly detd. on immunoblots where McC10 was found to exclusively recognize ***mouse*** kappa light chains and display no cross-reactivity with ***mouse*** Ig heavy chains nor with kappa light chains from ***rat*** or rabbit. In addn., McC10 was used successfully in two-step immunocytochem. (ICC) for the localization of enkephalin, nerve growth factor (NGF) receptor and paired helical

filament-immunoreactive sites in ***rat*** brain, ***rat*** skin and human brain, resp., using ***mouse*** IgG's and IgM's as primary antibodies. McC10 compared favorably with peroxidase-anti-peroxidase ICC with respect to sensitivity but was markedly superior with respect to specificity when used in fixed human brain or ***rat*** skin. This study demonstrates some of the potential advantages of using an epitope specific monoclonal bi-specific developing reagent like McC10 in an immunobased technique like ICC. Its potential use in a variety of other immunobased procedures is discussed.

L6 ANSWER 43 OF 49 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 89323408 MEDLINE
DOCUMENT NUMBER: 89323408 PubMed ID: 2473803
TITLE: An enzyme-linked immunosorbent assay for erythropoietin using monoclonal antibodies, tetrameric immune complexes, and substrate amplification.
AUTHOR: Wognum A W; Lansdorp P M; Eaves A C; Krystal G
CORPORATE SOURCE: Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, Canada.
SOURCE: BLOOD, (1989 Aug 1) 74 (2) 622-8.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198908
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19960129
Entered Medline: 19890829
AB We recently reported the development of several monoclonal antibodies (MoAbs) to native human erythropoietin (Ep). In the present study we have used the two antibodies with highest affinity to develop a two-sided or sandwich enzyme-linked immunosorbent assay (ELISA) to measure Ep in human serum. In this assay Ep is incubated in microtiter wells precoated with the first (IgE) anti-Ep ***antibody*** . Assay wells are then incubated with the second (IgG1) anti-Ep ***antibody*** , which is labeled noncovalently with the enzyme alkaline phosphatase (AP) by means of ***bispecific*** tetrameric ***antibody*** complexes consisting of IgG1 anti-Ep cross-linked to IgG1 anti-AP using ***rat*** MoAbs specific for ***mouse*** IgG1. Application of this noncovalent labeling procedure, in combination with substrate amplification, results in a detection sensitivity of 0.5 to 1.0 mU/sample (5 to 10 mU/mL), which makes this assay suitable for measuring normal serum Ep levels. The validity of this ELISA for quantitating Ep in biological fluids was demonstrated by the parallelism obtained between pure recombinant Ep dose-response curves and those obtained with plasma and serum from healthy donors and patients with various hematologic disorders. Normal plasma Ep levels detected with this ELISA ranged from 9 to 101 mU/mL with a mean of 32 +/- 23 (SD) mU/mL. Ep levels in sera from patients with polycythemia vera were in the low to normal range, whereas Ep levels in sera from patients with secondary polycythemia and patients with aplastic anemia were moderately to strongly elevated. These results demonstrate that the Ep-ELISA is a sensitive, reliable, and nonradioactive immunologic method for quantitating Ep levels and should prove useful in a variety of clinical and laboratory settings.

L6 ANSWER 44 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:133496 CAPLUS
DOCUMENT NUMBER: 110:133496
TITLE: Bi-specific antibodies, their production, and their use with targeting antibodies in kits and in treating neoplastic, viral, and parasitic diseases
INVENTOR(S): Gilliland, Lisa Kim; Clark, Michael Ronald; Waldmann, Herman
PATENT ASSIGNEE(S): National Research Development Corp., UK
SOURCE: Brit. UK Pat. Appl., 21 pp.
CODEN: BAXXDU

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| GB 2197323 | A1 | 19880518 | GB 1987-25812 | 19871104 |
| GB 2197323 | B2 | 19901031 | | |
| WO 8803566 | A1 | 19880519 | WO 1987-GB782 | 19871104 |
| W: AU, DK, JP, US | | | | |
| RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE | | | | |
| AU 8781568 | A1 | 19880601 | AU 1987-81568 | 19871104 |
| AU 616870 | B2 | 19911114 | | |
| EP 293405 | A1 | 19881207 | EP 1987-907124 | 19871104 |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| JP 01501201 | T2 | 19890427 | JP 1987-506498 | 19871104 |
| DK 8803532 | A | 19880905 | DK 1988-3532 | 19880627 |

PRIORITY APPLN. INFO.: GB 1986-26413 19861105
WO 1987-GB782 19871104

AB ***Bispecific*** antibodies having a 1st binding affinity for a T-cell receptor capable of activating killing and a 2nd binding affinity for Ig, and fragments thereof retaining the binding affinity of the whole mol., are useful in treating disease, esp. neoplastic, viral, and parasitic diseases. The cytotoxic mols. or fragments are targeted against selected target cells through use in vivo of antibodies or fragments specific for the target cells. Alternatively, the ***bispecific*** ***antibody*** mols. or fragments are combined in vitro with the targeting antibodies or fragments to form a conjugate which is then used in vivo in treating disease. Processes for fusing hybridomas and for selecting the polydomas which produce the ***bispecific*** antibodies are disclosed. A ***rat*** hybridoma producing monoclonal antibodies to human CD3 antigen was prepd., selection was made for myeloma light chain loss variants, and the cells were poisoned with iodoacetamide. A ***mouse*** hybridoma was prepd. producing monoclonal antibodies to ***rat*** IgG of an allotype different than that produced by the 1st hybridoma. This hybridoma was selected for a variant neg. in hypoxanthine-guanine phosphoribosyl transferase (HPRT-). The poisoned cells were fused with the HPRT- cells, and hybrid cells were cloned and selected for prodn. of the ***bispecific*** monoclonal ***antibody*** . Supernatant of hybridoma LHC49.18.2 showed improved percent target cell lysis over that of parental lines in an effector cell retargeting assay using ***rat*** anti-Thy-1 monoclonal antibodies as targeting antibodies.

L6 ANSWER 45 OF 49 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:133495 CAPLUS
DOCUMENT NUMBER: 110:133495
TITLE: ***Bispecific*** antibodies, their production and use in treating neoplastic, viral, and parasitic diseases
INVENTOR(S): Clark, Michael Ronald; Waldmann, Herman
PATENT ASSIGNEE(S): National Research Development Corp., UK
SOURCE: Brit. UK Pat. Appl., 24 pp.
CODEN: BAXXDU
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| GB 2197322 | A1 | 19880518 | GB 1987-25811 | 19871104 |
| GB 2197322 | B2 | 19901010 | | |
| WO 8803565 | A1 | 19880519 | WO 1987-GB781 | 19871104 |
| W: AU, DK, JP, US | | | | |
| RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE | | | | |

AU 8781569 A1 19880601 AU 1987-81569 19871104
AU 616871 B2 19911114
EP 289546 A1 19881109 EP 1987-907123 19871104
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
JP 01501200 T2 19890427 JP 1987-506497 19871104
DK 8803488 A 19880905 DK 1988-3488 19880624
PRIORITY APPLN. INFO.: GB 1986-26412 19861105
WO 1987-GB781 19871104

AB ***Bispecific*** antibodies are prepd. having a 1st binding affinity for a human T-cell receptor capable of activating killing and a 2nd binding affinity for target cells, characterized in that the 2 heavy chains are selected to mitigate the killing of human T-cells by the antibodies. Such cytotoxic mols. and their fragments retaining the binding affinities of whole mol. are useful in treating disease, particularly neoplastic, viral, and parasitic diseases. Processes for fusing hybridoma cells and for selection of polydomas producing ***bispecific*** antibodies are disclosed. A ***rat*** hybridoma producing monoclonal antibodies to human CD3 antigen was prepd., selection was made for myeloma light chain loss variants, and the cells were poisoned with iodoacetamide. A 2nd ***rat*** hybridoma producing monoclonal antibodies to ***mouse*** Thy-1 antigen was prepd. and then selected for a variant neg. in hypoxanthine-guanine phosphoribosyl transferase (HPRT-). The poisoned cells were fused with the HPRT- cells, and hybrid cells were cloned and selected for prodn. of the ***bispecific*** monoclonal ***antibody*** . Supernatant of hybridoma SHN20.12 showed improved percent target cell lysis over that of parental lines.

L6 ANSWER 46 OF 49 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 88091734 MEDLINE
DOCUMENT NUMBER: 88091734 PubMed ID: 3121901
TITLE: T-cell killing of target cells induced by hybrid antibodies: comparison of two ***bispecific*** monoclonal antibodies.
AUTHOR: Clark M R; Waldmann H
CORPORATE SOURCE: Department of Pathology, University of Cambridge, England.
SOURCE: JOURNAL OF THE NATIONAL CANCER INSTITUTE, (1987 Dec) 79 (6) 1393-401.
Journal code: 7503089. ISSN: 0027-8874.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198802
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19880217

AB Two different ***bispecific*** hybrid antibodies were generated by cell fusion of pairs of existing hybrid-myeloma cell lines. Both hybrid antibodies had similar specificity for human CD3 and ***mouse*** Thy-1 but differed in the isotypes of the immunoglobulin heavy chains. Hybrid HA2b/2b was a hybrid between a ***rat*** IgG2b (CD3) and a ***rat*** IgG2b anti-Thy-1, whereas HA2b/2c was a hybrid between the same ***rat*** IgG2b (CD3) and a ***rat*** IgG2c anti-Thy-1. Both hybrid antibodies were found to be very potent in inducing the killing of Thy-1-positive targets by human T-cell blasts, with the hetero-hybrid HA2b/2c showing a higher titer. T-cell blasts generated from resting peripheral blood mononuclear cells by a novel mitogenic ***antibody*** , YTH361, were exploited as effector cells. In addition to the CD3-dependent killing, the ***rat*** IgG2b anti-Thy-1 ***antibody*** and the hybrid ***antibody*** HA2b/2b but not the ***rat*** IgG2c anti-Thy-1 or the hybrid ***antibody*** HA2b/2c were also able to elicit ***antibody*** -dependent cell-mediated cytotoxicity (ADCC). This ADCC was inhibited by an anti-FcRlow (CD16) monoclonal ***antibody*** , which suggests that these effectors were K-cells. Toxicity toward the T-cell blast effector population was also observed, but in this instance the hetero-hybrid HA2b/2c had a lower cytotoxic

titer. In conclusion, mixed isotype hybrid antibodies may have some advantages for eliciting T-cell-mediated killing of tumor cell targets by exhibiting a better therapeutic ratio of target cell to effector cell cytotoxicity.

L6 ANSWER 47 OF 49 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1987:47058 BIOSIS
DOCUMENT NUMBER: BA83:26404
TITLE: ADVANTAGES OF ***BISPECIFIC*** HYBRIDOMAS IN ONE-STEP IMMUNOCYTOCHEMISTRY AND IMMUNOASSAYS.
AUTHOR(S): SURESH M R; CUELLO A C; MILSTEIN C
CORPORATE SOURCE: MED. RESEARCH COUNCIL LAB. OF MOLECULAR BIOLOGY, HILLS ROAD, CAMBRIDGE CB2 2QH, ENGLAND.
SOURCE: PROC NATL ACAD SCI U S A, (1986) 83 (20), 7989-7993.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A chemical selection procedure has been used to prepare a hybrid hybridoma cell line (P4C1) following fusion of two previously established hybridomas secreting antiperoxidase and antisubstance P, respectively. P4C1 secretes ***bispecific*** monoclonal ***antibody*** alongside the two parental antibodies, with no visible inactive heterologous heavy-light chain pairs. The ***bispecific*** monoclonal ***antibody*** is thus easy to purify in excellent yields. The advantage of its monovalency for one antigen and simultaneous binding of a marker enzyme has been explored for its potential use in competitive immunoassays. Its use in immunocytochemistry led to major improvements in sensitivity, signal-to-noise ratio, simplification of staining procedures, and ultrastructural preservation of subcellular elements. Particularly remarkable was that, unlike conventional procedures, the immunoreaction with the ***bispecific*** monoclonal ***antibody*** was homogeneously distributed across the entire thickness of a 50-.mu.m section.

L6 ANSWER 48 OF 49 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 86247792 MEDLINE
DOCUMENT NUMBER: 86247792 PubMed ID: 3459660
TITLE: Cyclic tetramolecular complexes of monoclonal antibodies: a new type of cross-linking reagent.
AUTHOR: Lansdorp P M; Aalberse R C; Bos R; Schutter W G; Van Bruggen E F
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1986 Jun) 16 (6) 679-83.
Journal code: 1273201. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198607
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860730
AB A simple and efficient procedure for the construction of bifunctional molecules is described and their use in a variety of applications documented. This procedure is based on our observation that ***mouse*** IgG1 monoclonal antibodies, when mixed with equimolar amounts of a high-affinity ***rat*** monoclonal ***antibody*** specific for ***mouse*** IgG1, yield uniform cyclic tetramolecular complexes each consisting of two ***mouse*** and two ***rat*** antibodies as shown by gel electrophoresis and electron microscopy. When solutions of two ***mouse*** antibodies (e.g. a and b) are mixed prior to the formation of complexes with the ***rat*** ***antibody*** , stable ***bispecific*** (a X b) complexes together with monospecific (a X a and b X b) complexes are obtained. ***Bispecific*** complexes prepared in this way were able to efficiently bind peroxidase to cell surface antigens, and to bind red blood cells to selected nucleated cell types present in heterogeneous populations. Tetrameric ***antibody*** complexes are more easily prepared than ***bispecific*** antibodies or

bifunctional antibodies produced by transfection of myelomas with recombinant genes. They also have the advantage that the antigen-binding properties of the bivalent monoclonal antibodies are not compromised. Tetrameric ***antibody*** complexes thus represent a powerful new type of cross-linking reagent that may have a wide spectrum of applications in biology and medicine.

L6 ANSWER 49 OF 49 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 83240096 EMBASE
DOCUMENT NUMBER: 1983240096
TITLE: Hybrid hybridomas and their use in immunohistochemistry.
AUTHOR: Milstein C.; Cuello A.C.
CORPORATE SOURCE: MRC Lab. Mol. Biol., Cambridge CB2 2QH, United Kingdom
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AB A normal ***antibody*** -producing cell only expresses one ***antibody*** , resulting in the well-known phenomenon of allelic exclusion. When two myeloma cells are fused, the derived hybrids are capable of co-dominantly expressing the ***antibody*** genes of both parents. Although the respective variable (V) and constant (C) region genes remain expressed in the same cis configuration, heavy and light chains of both parents are scrambled, and hybrid molecules are formed. The same is true when a myeloma and an ***antibody*** -producing cell are fused to produce a hybrid myeloma (hybridoma). Fusion therefore allows the production of hybrid immunoglobulin molecules containing two different combining sites. Hybrid molecules of this type retain antigen-binding activity and specificity. ***Bispecific*** monoclonal antibodies secreted by hybridomas may have a variety of uses in biology and in medicine. Here we have focused on their application in histochemistry. As an example, er have prepared and tested an anti-somatostatin-anti-peroxidase ***bispecific*** ***antibody*** . This way of producing hybrid molecules is superior to the production of hybrid antibodies by chemical reconstitution methods because the drastic treatment required for chain separation in the latter is likely to lead to some protein denaturation and loss of ***antibody*** activity. Intracellularly synthesized and assembled hybrids do not suffer from this disadvantage. In addition, the recombination of heavy and light chains from different ***antibody*** molecules is likely to lead to considerable waste.